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Detection and plasma pharmacokinetics of an anti-vascular endothelial growth factor oligonucleotide-aptamer (NX1838) in rhesus monkeys

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Abstract

Aptamers are oligonucleotide ligands selected, in vitro, to bind a specified target protein. The first aptamer to reach human clinical testing is NX1838, a polyethylene glycol conjugated aptamer that inhibits vascular endothelial growth factor. This paper describes the validation of a high-performance liquid chromatographic anion-exchange method for the determination of NX1838 in plasma. Measurements of intact NX1838 had a coefficient of variation of less than 8% and an accuracy between 107% and 115%. The assay was utilized to determine NX1838 plasma pharmacokinetics in rhesus monkeys following a single 1 mg/kg intravenous or subcutaneous dose. Following intravenous administration, the maximum achieved plasma concentration was 25.5 μg/ml with a terminal half-life of 9.3 h and clearance rate of 6.2 ml/h. After subcutaneous administration, the fraction of the dose absorbed into the plasma compartment was 0.78 with a time to peak concentration (4.9 μg/ml) of 8 to 12 h.

Keywords: Aptamer; NX1838

1. Introduction

Nucleic acid based aptamers are oligonucleotide ligands that are selected to bind to specific target proteins. Aptamers are identified via the Systematic Evolution of Ligands by EXponential enrichment (SELEX) process which allows the rapid isolation, in vitro, of the few oligonucleotide ligands, from a random-sequence pool of approximately 10^{15} sequences, that bind a particular target molecule with high affinity and specificity [1–6]. The starting library of nucleic acid sequences can be chemically modified to enhance the nuclease stability of the resultant aptamers [7–9]. Once isolated, aptamers can be "post-SELEX" chemically modified and/or conjugated to a polyethylene glycol (PEG) chain, lipid, lipoprotein or liposome to further enhance their pharmacokinetic properties [10–13].

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NX1838 (a PEG-conjugated aptamer) is an inhibitor of vascular endothelial growth factor (VEGF)/vascular permeability factor [12]. The aptamer portion of NX1838 is a modified synthetic oligonucleotide, 27 nucleotides in length, in which the 2′-position of the ribose sugar ring of all pyrimidines consists of a fluorine moiety and the same position of all but two purines consists of an 2′-*O*-methyl group [12]. NX1838 also contains a 40 kD PEG molecule conjugated to the 5′-terminus of the aptamer and a deoxythymidine linked to the 3′-terminus via a 3′–3′ linkage [12].

Angiogenesis, the growth of new blood vessels, is a necessary process in adults for normal physiological processes such as wound healing and menstruation [14]. However, angiogenesis is an important factor in pathophysiologic conditions such as the growth of solid tumors or the severe loss of vision in macular degeneration or diabetic retinopathy [15,16].

VEGF has been shown to be an endothelial cell mitogen and chemoattractant in vitro [17,18] and to induce angiogenesis and vascular permeability in vivo [19,20]. Inhibition of VEGF function can inhibit the growth of solid tumors in experimental animal models [21,22] and elevated levels of VEGF are correlated with ocular neovascularization [23–25]. Because of these observations, VEGF is implicated as a stimulator of angiogenesis and has become a target for therapeutic efforts aimed at blocking angiogenesis in pathological conditions.

Four different isoforms of VEGF have been identified. Each isoform ($VEGF_{165}$ $VEGF_{121}$, $VEGF_{189}$ and $VEGF_{206}$) occurs as the result of alternative splicing events and each isoform is secreted as a disulfide linked homodimer [26]. Examination of simian cDNA's revealed the VEGF protein sequences to be identical to their human homologue [27]. NX1838 specifically recognizes $VEGF_{165}$, the major soluble form of VEGF, and can block $VEGF_{165}$ binding to both FLT-1 and KDR VEGF-receptors in vitro [12]. In animal models, NX1838 can inhibit both vascular permeability and the growth of solid tumors [12,28].

NX1838 is currently in phase I human trials for the treatment of macular degeneration, a progressive loss of vision due to abnormal angiogenesis into and surrounding the retina. This is the first aptamer-based therapeutic to reach human testing and in support of these trials it is necessary to investigate the plasma levels in humans and to understand the systemic plasma pharmacokinetics of the compound in a primate model. Therefore, a HPLC analytical assay method was developed to quantify NX1838 in human and rhesus monkey plasma samples. The validated method was utilized to investigate the plasma pharmacokinetics following a single intravenous or single subcutaneous dose of NX1838 in rhesus monkeys.

2. Experimental

2.1. NX1838

The synthesis of NX1838 has been described previously [12]. The aptamer portion, 5′-CGGAAUCAGUGAAUGCUUAUACAUCCG-3′, is identical to the sequence, designated t44-OMe, described by Ruckman et al. [12]. The two adenosine listed in bold type are the only two unmodified ribose nucleotides.

NX1838 contains a 40 kD PEG moiety. For peak identity experiments, versions of NX1838 were synthesized that did not contain a PEG-moiety or which contained a 20 kD PEG in place of the standard 40 kD-PEG conjugate.

2.2. Sample preparation

Human and monkey ethylenedinitrilo)-tetraacetic acid (EDTA) plasma samples were frozen at −80°C until use. After allowing each sample to thaw, the plasma was mixed and an aliquot (100 μl) transferred to a 1.7 ml polypropylene tube. Methanol (200 μl) was added to each sample and, after mixing, the plasma protein was removed by centrifugation at 14 000 *g* for 10 min. An aliquot (150 μl) of the supernatant was transferred to a 250 μl limited volume insert vial for HPLC analysis.

2.3. Equipment

A Waters Alliance HPLC system consisting of a 2690 separations module (containing pumps and autosampler), external column heater, model TCM column heater controller and model 916 UV detector

was used for sample analyses. Separations were performed at a flow-rate of 1 ml/min with a Dionex Nucleopac PA100 guard-column (4×50 mm) and a Dionex Nucleopac TM PA-100 column (4×250 mm) connected in series.

2.4. Column liquid chromatography

Mobile phase A consisted of a 0.7% v/v acetonitrile–water mixture buffered at pH 8.0 (ambient temperature) using 17 m*M* Tris(hydroxymethyl)aminomethane. Mobile phase B was identical to A except that 2 *M* lithium chloride was added. The HPLC column was heated to 80°C and equilibrated with 100% mobile phase A. Following injection of 100 μl of sample, a linear gradient (0% to 35% mobile phase B in 8 min) was begun. For peak identity experiments, the gradient was extended for an additional 3 min.

2.5. Assay validation

NX1838 calibration standards were prepared in human EDTA-plasma. EDTA was chosen as the anticoagulant due to its nuclease inhibitory properties. Quality control solutions were prepared both in human and in rhesus monkey EDTA-plasma. For each standard or control solution, several individual 125 μl aliquots were made and stored frozen at −20°C until use. For analysis, standards and quality control solutions were thawed, and 100 μl aliquots processed in parallel with the test samples. Calibration standard data (peak area versus concentration) were fit by linear regression using a variance-stabilizing transformation [29].

2.6. Linearity and range

Five samples of each of the 8 calibration standards (0.13 to 24.9 μg NX1838/ml plasma) were assayed and the estimated value for each determined from an independent standard curve. The concentrations of standards and samples are expressed as aptamer (oligonucleotide) weight only and are based on an approximate extinction coefficient for the aptamer of 37 $\mu g/ml/A_{260}$ unit.

2.7. Accuracy and precision

Accuracy and precision was assessed by determining the mean, coefficient of variation (C.V.) and percent Bias (%Bias) for the NX1838 quality control samples as determined on two different days by two different operators. Each operator analyzed six replicates of each quality control sample on each day for a total of 24 replicates for each quality control sample.

2.8. Stability

For freeze/thaw stability analyses, triplicate aliquots of NX1838 quality control samples were subjected to three freeze/thaw cycles prior to analysis. The mean value of each quality control concentration was compared to the corresponding mean value previously obtained without any additional freeze/thaw cycles. For room temperature stability, duplicate aliquots of NX1838 quality control samples were thawed and incubated at room temperature for 18 h prior to analysis. The mean value of each quality control sample was compared to the corresponding mean previously obtained. For freezer stability, triplicate aliquots of NX1838 quality control samples were assayed after more than two months of storage at −20°C. Concentrations were determined using calibration standards prepared on the day of analysis (not frozen). The mean of each quality control sample was compared to the corresponding mean previously obtained.

2.9. Animal protocols

Female rhesus monkeys (Macaca *mulatta*) were obtained and cared for in accordance with all applicable state and federal guidelines and adhered to the "Principles of Laboratory Animal Care" (NIH publication #85-23, revised 1985). Body weights were measured prior to dosing (range 3.4–5.6 kg) and the dose volume adjusted for each animal to achieve 1 mg of aptamer per kg of body weight (1 mg/kg). The same set of six female monkeys was utilized for both the intravenous (I.V.) and subcutaneous (SubQ) groups. Drug solution was prepared on the day of dosing (2 mg/ml based on oligonucleotide weight) in phosphate buffered saline pH=6.4. The I.V. ex-

periment, which was performed first, was begun with a single bolus injection made through the saphenous vein. After allowing 16 days to clear the I.V. administered compound, the SubQ experiment was performed beginning with a single injection in the back. For blood draws, animals were restrained by a squeeze-back cage mechanism in order to minimize injury, movement and stress. Blood samples (approximately 500 μl) for both experiments were obtained in EDTA-containing tubes by direct venipuncture of the femoral vein. Samples were centrifuged at 1500 *g* for 10 min and the plasma fraction immediately frozen at −70°C. A plasma sample was obtained for all animals immediately prior to dosing and plasma samples for each animal were obtained at 5 min, 15 min, 30 min, and 1, 2, 4, 8, 16, 24, 32, and 72 h after administration of dose.

2.10. Pharmacokinetic analyses

Pharmacokinetic parameters were determined using non-compartmental analyses (WinNonlin, version 1.5). Data were plotted and analyzed for each animal in the study and the resulting pharmacokinetic parameters averaged. The following parameters were calculated for both dose groups: Maximum plasma concentration (Cmax); area under the curve extrapolating to infinite time (AUC); elimation half-life (Elim.T1/2); plasma clearance (Cl); fraction of dose absorbed [(F) as calculated for the subQ group as the quotient resulting from the AUCINF of the subcutaneous group divided by the AUCINF of the I.V. group]. The volume of distribution at steady state (Vss) was calculated only for the I.V. group while the time to maximum plasma concentration (Tmax) was calculated only for the SubQ group.

3. Results

To support both pre-clinical and clinical development of NX1838, an assay for both human and rhesus monkey EDTA-plasma samples was necessary. We chose to validate the assay for human plasma and then to cross validate the assay for rhesus monkey plasma. Fig. 1 illustrates representative HPLC chromatograms for both human and rhesus monkey plasma with and without the presence of

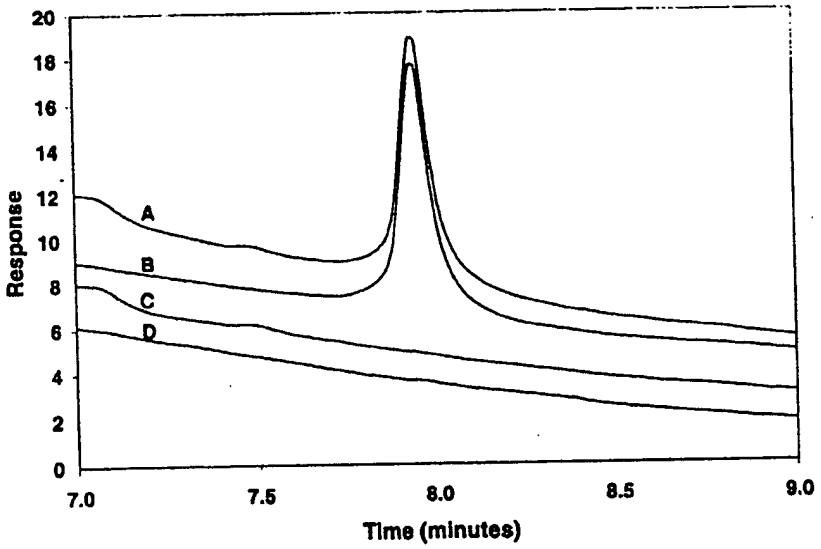

Fig. 1. HPLC chromatograms of medium quality control sample (2.07 μg/ml) in pooled human plasma (A), medium quality control sample (2.07 μg/ml) in pooled rhesus monkey plasma (B), blank pooled human plasma (C), and blank pooled rhesus monkey plasma (D). Chromatograms are artificially staggered to better display the data. The unit of response is a millivolt at 270 nm.

NX1838. Neither matrix presented peaks that interfered with the highly anionic NX1838.

3.1. Linearity and range in human plasma

For linearity and range, 5 sets (A–E) of calibration standards were analyzed and the concentration of each standard determined from a calibration curve generated from an independent set of calibration standards. Data from these sample sets are shown in Table 1 along with the calculated mean, percent C.V. (%C.V.), and percent bias (%Bias) at each concentration level. At all concentrations, the mean %Bias values determined by the assay were within ±8.5% of the expected value (−5.4% to +8.5%). The precision, as expressed by the %C.V., was 6.7% or lower. Ordinary linear regression (not shown) of the observed mean concentrations versus the known concentrations yielded an r^2 of 0.9994 with a slope of 1.05 (95% confidence interval 1.04 to 1.06) and a y-intercept of −0.046 (95% confidence interval −0.13 to 0.041). These results demonstrated an effective range of 0.13 to 24.9 μg/ml.

3.2. Precision and accuracy (human plasma)

For precision and accuracy determinations, human pooled plasma was spiked to create three different known concentrations of NX1838 [designated the low quality control sample (LQC), high quality control sample (HQC), and middle quality control sample (MQC)]. Two operators on each of two different days assayed six replicates from each concentration. The data are shown in Table 2. The overall variability for each NX1838 quality control concentration was small (%C.V.≤7.6%) and the overall accuracy, as expressed by the %bias, for each quality control concentration was within 14.5%. Variance component analysis was performed at each concentration to quantify the proportion of variability due to inter-day, inter-operator, and intra-assay effects. This analysis estimated that the majority of error stems from intra-assay variability except for the LQC in which the error was fairly equally distributed between the inter-day, inter-operator and intra-assay variability.

3.3. Specificity in human plasma

Results of specificity analyses show no interfering peaks at the expected retention time of NX1838 for any of the six tested individual blank human EDTA-plasma samples or for the pooled human EDTA-plasma used to make the calibration standards and quality control samples (Fig. 1 and data not shown).

3.4. Stability in human plasma

NX1838 was found to be stable in human EDTA-plasma after repeated freeze/thaw cycles (3×), after incubation at ambient temperature for 18 h or after storage at −20°C for over two months. The mean values obtained for each quality control concentration after an ambient temperature incubation were 0.23 μg/ml for the LQC, 2.30 μg/ml for the MQC and 24.8 μg/ml for the HQC, all within 4.7% of the

Table 1
Linearity and range. Standards prepared in human plasma

Set	Concentration of NX1838 (μg/ml)							
	0.13	0.26	0.52	1.04	2.48	4.97	12.4	24.9
A	0.15	0.26	0.53	1.03	2.50	4.97	13.6	25.6
B	0.13	0.24	0.51	1.02	2.45	5.01	13.4	25.6
C	0.13	0.24	0.52	1.01	2.45	4.93	13.4	26.2
D	0.13	0.24	0.51	1.00	2.44	4.99	13.6	26.1
E	0.13	0.25	0.49	0.99	2.45	4.94	13.4	26.1
Mean	0.13	0.25	0.51	1.01	2.46	4.97	13.5	25.9
Std. Dev.	0.009	0.009	0.015	0.016	0.024	0.033	0.11	0.29
%C.V.	6.7%	3.6%	2.9%	1.6%	1.0%	0.66%	0.8%	1.1%
%Bias	3.1%	−5.4%	−1.5%	−2.9%	−0.9%	−0.04%	8.9%	4.0%
N	5	5	5	5	5	5	5	5

Table 2
Precision and accuracy, human plasma

Day	Operator	Concentration of NX1838 (μg/ml)		
		0.207 (LQC)	2.07 (MQC)	20.7 (HQC)
1	1	0.23	2.25	23.8
		0.24	2.27	23.8
		0.25	2.27	24.0
		0.24	2.28	24.0
		0.23	2.31	23.9
		0.24	2.26	24.0
2	1	0.24	2.26	23.3
		0.22	2.20	23.5
		0.23	2.21	23.4
		0.22	2.20	23.2
		0.21	2.19	23.3
		0.23	2.24	23.2
1	2	0.23	2.22	23.7
		0.22	2.22	23.3
		0.23	2.18	23.2
		0.22	2.20	23.7
		0.23	2.25	23.7
		0.22	2.19	23.5
2	2	0.19	2.23	24.1
		0.19	2.17	23.9
		0.20	2.20	24.1
		0.21	2.25	24.1
		0.19	2.21	24.0
		0.21	2.21	23.5
Mean		0.22	2.23	23.7
Std. Dev.		0.017	0.095	0.32
%C.V.		7.6%	1.6%	1.4%
%Bias		7.1%	7.6%	14.5%
N		24	24	24

means as determined for each sample concentration without an ambient temperature incubation. Likewise, the mean for each quality control concentration after 3 freeze/thaw cycles were 0.23 μg/ml for the LQC, 2.22 μg/ml for the MQC, and 23.7 μg/ml for the HQC; all within 4.5% of the means as determined without additional freeze/thaw cycles. Finally, when frozen quality control samples (71 days at −20°C) were assayed utilizing a freshly prepared, never frozen, set of calibration standards, the mean values for the LQC (0.22 μg/ml) the MQC (2.32 μg/ml) and the HQC (24.7 μg/ml) differed by 4.3% or less from the baseline values (Table 2). These results established suitable stability parameters for routine sample collection, handling, and storage.

3.5. Rhesus monkey cross validation

To cross validate the accuracy, precision and stability parameters of the assay for rhesus monkey EDTA-plasma samples, rhesus monkey pooled EDTA-plasma was spiked to create three different known concentrations of NX1838 (LQC, HQC and MQC). Three replicates from each concentration were assayed using the calibrators made in human EDTA-plasma. Results are summarized in Table 3 and show that the overall variability (C.V.<1.5%) and accuracy (%bias<13.5%) was similar to that observed for the human samples.

Analyses of blank EDTA-plasma samples obtained from five individual rhesus monkeys (data not shown) as well as pooled EDTA-plasma used for preparation of the quality control samples (Fig. 1D) revealed no interfering peaks at the expected retention time of NX1838. Repeated freeze/thaw and bench top stability parameters were similar to that observed for human samples (data not shown).

3.6. Plasma pharmacokinetics of single dose I.V. or SubQ administration of NX1838

The assay was applied to the analysis of rhesus monkey plasma pharmacokinetics following a single 1 mg/kg I.V. or 1 mg/kg SubQ administration of NX1838. Fig. 2 shows the mean NX1838 plasma concentration versus time profile for both dosing regimens and Tables 4 and 5 summarize the non-compartmental pharmacokinetic parameters obtained

Table 3
Cross validation of rhesus monkey plasma

QC	NX1838 (μg/ml) (mean; C.V.)			N
	LQC	MQC	HQC	
Human EDTA-plasma QC's (baseline)	0.23; 0%	2.21; 2.0%	23.6; 0.37%	3
Rhesus monkey EDTA-plasma QC's	0.21; 0%	2.15; 1.4%	23.5; 0.37%	3
Percent deviation from baseline	−8.7%	−2.7%	−0.4%	

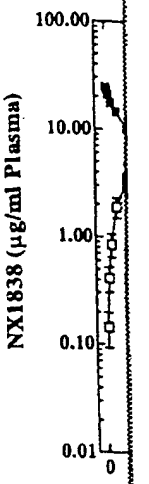

Fig. 2. Rhesus
of NX1838 fol
neous adminis
terminations wi
standard error

Table 4
Pharmacokinetic

Animal number	
Parameter	
Cmax	
AUCinf	
Elim.T1/2	
Cl	
F	
Vss	

Cmax=maxi
of dose absorb

Table 5
Pharmacokinetic

Animal number	
Parameter	
Tmax	
Cmax	
AUCinf	
Elim.T1/2	
Cl/F	
F	

Tmax=time
elimation half-l

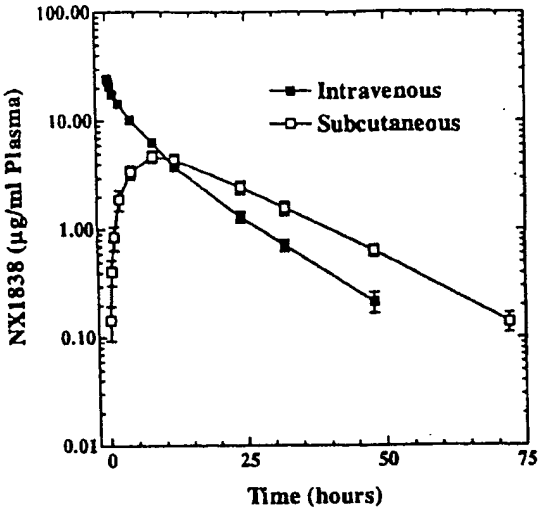

Fig. 2. Rhesus monkey plasma concentration versus time curves of NX1838 following a single 1 mg/kg intravenous or subcutaneous administration. Data are shown as the mean of six determinations with a point to point fit applied. Error bars represent standard error of the mean.

for each animal in each dose group as well as the mean values and associated standard deviations.

Estimates for Cmax were 25.5 µg/ml at time zero by I.V. administration and 4.9 µg/ml (8 to 12 h post administration) by subcutaneous administration. Estimated elimination half-lives were 9.3 h for I.V. administration and 12 h for subcutaneous administration. The AUC for the subcutaneous group was 78% of the AUC observed for the I.V. group (128.5 µg h/ml versus 165 µg h/ml). The Vss for the I.V. group averaged 60 ml/kg.

3.7. Peak identity

Although the HPLC retention times obtained for the NX1838 peak for the standards, QC's and test samples were identical, the question remained whether this peak represented full length NX1838. The presence of the PEG moiety on NX1838 made direct measurement of the mass of the HPLC peak impossible. Therefore, to determine if this peak

Table 4
Pharmacokinetic parameters for NX1838 following a single 1 mg/kg intravenous dose

Animal number		5319 value	5746 value	5785 value	5896 value	6251 value	6788 value	Mean value	S.D. value
Parameter	Units								
Cmax	(µg/ml)	20.8	27.1	26.8	26.3	26.6	25.4	25.5	2.4
AUCinf	((µg h)/ml)	161	177	138	204	153	155	165	22.9
Elim.T1/2	(h)	8.7	9.6	6.7	9.6	10.7	10.7	9.3	1.5
Cl	(ml/(h kg))	6.2	5.6	7.2	4.9	6.5	6.5	6.2	0.8
F		1	1	1	1	1	1	1	–
Vss	(ml/kg)	60	52	53	54	70	67	60	7.6

Cmax=maximum plasma concentration; AUC=area under the curve; Elim.T1/2=elimation half-life; Cl=plasma clearance; F=fraction of dose absorbed; Vss=volume of distribution at steady state; S.D.=standard deviation.

Table 5
Pharmacokinetic parameters for NX1838 following a single 1 mg/kg subcutaneous dose

Animal number		5319 value	5746 value	5785 value	5896 value	6251 value	6788 value	Mean value	S.D. value
Parameter	Units								
Tmax	(h)	8.0	12.0	8.0	12.0	8.0	8.0	9.3	2.1
Cmax	(µg/ml)	5.6	3.4	5.6	7.1	3.8	3.6	4.9	1.5
AUCinf	((µg h)/ml)	139.3	113.3	123.6	186.7	120.7	87.5	128.5	33.2
Elim.T1/2	(h)	12.3	12.5	12.0	10.4	12.5	12.2	12.0	0.8
Cl/F	(ml/(h kg))	7.2	8.8	8.1	5.4	8.3	11.4	8.2	2.0
F		0.86	0.64	0.89	0.92	0.79	0.57	0.78	0.14

Tmax=time to maximum plasma concentration; Cmax=maximum plasma concentration; AUC=area under the curve; Elim.T1/2=elimation half-life; Cl=plasma clearance; F=fraction of dose absorbed; S.D.=standard deviation.

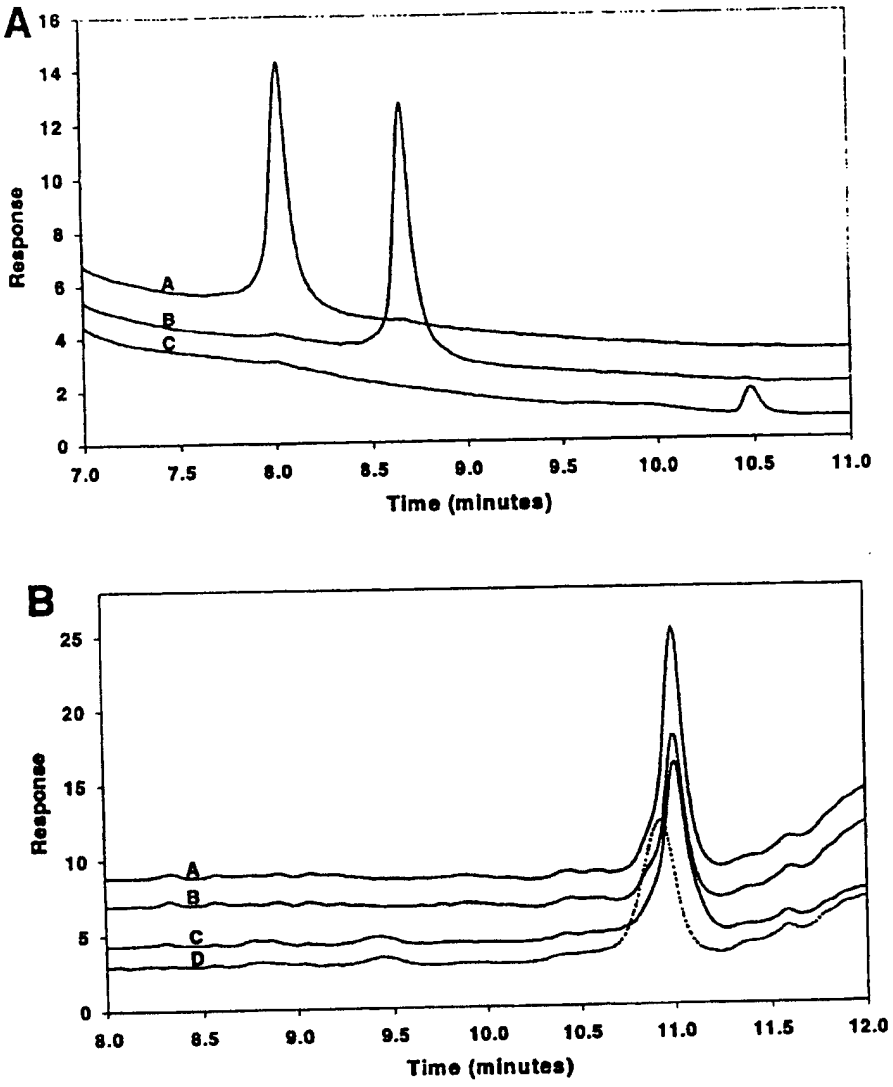

Fig. 3. Specificity of the NX1838 HPLC assay. Panel A. HPLC chromatograms following extraction from human plasma of NX1838 (containing the standard 40 kD PEG moiety) (A), NX1838 containing a 20 kD PEG in place of the normal 40 kD PEG (B) or NX1838 minus any conjugated PEG (C). Panel B. HPLC chromatograms of the base hydrolyzed 23-nucleotide fragment obtained from a 12-h time point following I.V. administration (A), a 12-h time point following subcutaneous administration (B) or the base hydrolyzed NX1838 standard (C). Base hydrolyzed NX1838 standard following phosphorylation of the 5′-termini is also shown (D). For base hydrolysis, isolated peaks and standards were exchanged into 50 m*M* sodium carbonate pH=9.6 and heated, in a capped tube, on a dry heat block (124°C) for 30 min. Chromatograms are artificially staggered to better display the data. The unit of response is a millivolt at 270 nm.

represented full length NX1838, two experiments were performed.

The first experiment was to determine the effect of PEG deletions on peak recovery and retention time. NX1838 minus PEG or with a 20 kD-PEG in place of the standard 40 kD-PEG were separately spiked into human EDTA-plasma. Recovery of these molecules were compared to NX1838 (containing the

standard 40 kD-PEG) spiked into plasma at an equivalent concentration. As shown in Fig. 3A, the 20 kD-PEG version was completely recovered during the extraction (100.3% recovery), but eluted 38 s after NX1838. Thus, unless significantly more than a 20 kD PEG was attached, the assay could not have quantified the compound.

The minus-PEG version of NX1838 did not elute with the standard assay gradient. However, by extending the gradient, the retention time was determined to be 2.47 min later than NX1838. The low recovery of the minus-PEG version of NX1838 (7.9%) was probably because the aptamer largely precipitated during the methanol extraction. The shorter retention times of the PEG-conjugated aptamers on the anion-exchange column was presumably due to the PEG moieties hindering association between the negative charges on the aptamer and the resin.

The second set of experiments was to determine if the 3′-terminal portion of the NX1838 peak was intact. Here, a NX1838 standard was base hydrolyzed along with the isolated NX1838 peaks obtained from 12-h rhesus monkey plasma samples from both dose regimens. Because there are only two ribose nucleotides, complete hydrolysis should yield the 3′-terminal 23 nucleotide fragment (including the 3′–3′ cap) that is not coupled to PEG. Hydrolysis fragments obtained from the standard and 12 h plasma samples had the same retention time by HPLC analysis (Fig. 3B). Analysis of the standard fragment following phosphorylation of the 5′-termini using polynucleotide kinase, showed a shift in retention time demonstrating the resolution of the method was sufficient to see charge differences (Fig. 3B). These results demonstrate that the NX1838 peak contains an intact 3′-terminus.

4. Discussion

An analytical HPLC method for the determination of a novel aptamer compound has been developed and validated for both human and rhesus monkey EDTA-plasma samples. The assay is simple, rapid and reproducible although an improvement in detection limit would be desirable to facilitate analyses beyond the time frame of the experiments presented here or for lower dose regimens. For example, all of the 72 h time points for the I.V. dose group were below the assays limit of quantification (0.13 μg/ml). It was also demonstrated that the NX1838 HPLC peak consisted of the full-length aptamer conjugated to PEG.

With the advent of antisense, ribozyme and nucleic acid-aptamer strategies for the treatment of disease, the interest in the pharmacokinetics of nucleic acids has increased sharply in recent years. A greater understanding of the pharmacokinetics may lead to methods to maximize the potential of these new therapeutic agents. The use of primate models is especially important as a prelude to designing dosing regimes in human clinical trials.

Previous studies with nucleic acid based therapeutics indicate that unmodified oligonucleotides are eliminated quickly via nuclease digestion. The addition of protecting groups at the 2′-position of the sugar ring and the use of inverted 3′–3′ caps demonstrated a substantial reduction in nuclease susceptibility [7–9] and the addition of large molecular weight conjugates reduced the clearance, in rats, significantly [11,13]. This latter effect is presumably the result of reduced kidney filtration.

In general, the plasma pharmacokinetic parameters determined for the I.V. administration of NX1838 in rhesus monkeys were similar to those obtained for Sprague Dawley rats at an identical 1 mg/kg dose (S.C.G. unpublished observation). The clearance rate determined for rats [7.8 ml/(h kg)] and the elimination $t_{1/2}$ (6 h) were similar to the clearance [6.2 ml/(h kg)] and elimination $t_{1/2}$ (9.3 h) obtained in monkeys. These observations support similar overall properties of plasma clearance of NX1838 between species.

The Vss calculated from the I.V. group averaged 60 ml/kg or slightly greater than the expected plasma volume (44.8 ml/kg) of a rhesus monkey [30]. The low value observed for Vss suggests that NX1838 is primarily confined to the systemic circulation.

The time to peak plasma concentrations (Tmax) following SubQ administration occurred 8–12 h following administration of the dose and 78% of the dose entered the plasma compartment (Table 5) indicating that the majority of NX1838 was systemically absorbed. NX1838 concentrations were

maintained above 15 nM throughout the 72-h time course of the study. Although the plasma level of $VEGF_{165}$ in rhesus monkeys is not known, circulating levels of $VEGF_{165}$ in humans ranges from 20 to 141 pg/ml (0.4 to 2.8 pM) in normal plasma and from 32 to 418 pg/ml (0.6 to 8.4 pM) in the plasma of cancer patients [31]. The estimated dissociation constant (Kd) of NX1838 for human $VEGF_{165}$ is 200 pM [12]. Thus NX1838 plasma levels were maintained at over 75 times the Kd and well above the likely plasma concentration for $VEGF_{165}$ during the entire three-day course of this study. Therefore, subcutaneous administration may be an effective option for the systemic delivery of aptamers.

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